185.(New) The method of Claim 184, wherein the diagnostic agent is an imaging or contrast agent.

186.(New) The method of Claim 184, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

187.(New) The method of Claim 178, wherein the medicant is a N-methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

188.(New) The method of Claim 187, wherein the viral vector is a therapeutic adenoviral or herpes simplex virus vector.

189.(New) The method of Claim 178, wherein the abnormal brain region is a glioma or ischemic brain region.--.

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## REMARKS

The Pending Claims

Before entry of the amendments contained herein, Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 are pending in the application. Claims 1-10 and 12-24 are directed to a method of delivering a medicant to an abnormal brain region in a mammalian subject. Claims 48-55 and 57-71 relate to a method of delivering a medicant to a malignant tumor in a mammalian subject. Claims 135-144 and 146-152 relate to a pharmaceutical composition.

Claims 153-160 are directed to a kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor.

Applicant's Amendment

The amendments to the specification submitted herein are to correct obvious typographical errors, and at page 19, line 4, to insert a SEQ ID NO for the nonopeptide sequence of bradykinin, in compliance with 37 C.F.R. §§ 1.821-1.825. A paper copy of a computer readable form sequence listing and a letter and statement under 37 C.F.R. §§ 1.821(e) and (f) are submitted herewith.

The amendment to Claims 1, 48, 135, and 153 reciting ". . . an in vivo activator of calcium-activated potassium channel . . ." is supported in the specification, as originally filed, e.g., at page 9, lines 12-14; and at page 13, line 28 through page 14, line 7. The amendments to Claims 1, 18-24, 48, 65-71, 135-137, and 153 reciting "said *activator* " instead of "said potassium channel activator" are merely for greater conciseness and to comport with the antecedent basis in view of the claim amendments noted in the previous paragraph.

New Claims 162-189 are added:

Support for new Claim 162 is found in the specification, for example, at page 14, lines 2-7; at page 16, line 21 through page 17, line 20; at page 26, lines 15-18; in Example 2, at page 30, lines 11-14; at page 31, lines 4-14; Figures 1-3; and Claims 1-3, 5, and 10, as originally filed.

Support for new Claim 163 is found, e.g., in Claim 10, as originally filed.

Support for new Claim 164 is found, e.g., in Claim 18, as originally filed.

Support for new Claim 165 is found, e.g., in Claim 19, as originally filed.

Support for new Claim 166 is found, e.g., in Claim 20, as originally filed.

Support for new Claim 167 is found, e.g., in Claim 21, as originally filed.

Support for new Claim 168 is found, e.g., in Claim 22, as originally filed.

Support for new Claim 169 is found, e.g., in Claim 23, as originally filed.

Support for new Claim 170 is found, e.g., in Claim 24, as originally filed.

Support for new Claim 171 is found, e.g., in Claim 12, as originally filed.

Support for new Claim 172 is found, e.g., in Claim 13, as originally filed.

Support for new Claim 173 is found, e.g., in Claim 14, as originally filed.

Support for new Claim 174 is found, e.g., in Claim 15, as originally filed.

Support for new Claim 175 is found, e.g., in Claim 16, as originally filed.

Support for new Claim 176 is found, e.g., in Claim 17, as originally filed.

Support for new Claim 177 is found, e.g., in Claims 2-3, as originally filed.

Support for new Claim 178 is found in the specification, for example, at page 14, lines 2-7; at page 16, line 21 through page 17, line 20; at page 26, lines 15-18; in Example 2, at page 30, lines 11-14; at page 31, lines 4-14; Figures 1-3; and Claims 1-3, 5, and 10, as originally filed.

Support for new Claim 179 is found, e.g., in Claim 10, as originally filed.

Support for new Claim 180 is found, e.g., in Claim 18, as originally filed.

Support for new Claim 181 is found, e.g., in Claim 19, as originally filed.

Support for new Claim 182 is found, e.g., in Claim 20, as originally filed.

Support for new Claim 183 is found, e.g., in Claim 12, as originally filed.

Support for new Claim 184 is found, e.g., in Claim 13, as originally filed.

Support for new Claim 185 is found, e.g., in Claim 14, as originally filed.

Support for new Claim 186 is found, e.g., in Claim 15, as originally filed.

Support for new Claim 187 is found, e.g., in Claim 16, as originally filed.

Support for new Claim 188 is found, e.g., in Claim 17, as originally filed.

Support for new Claim 189 is found, e.g., in Claims 2-3, as originally filed.

The Office Action and Applicant's Response

The Examiner acknowledged Applicant's election of designated claim Group I in Paper No. 7, which Applicant mailed January 22, 2002. However, in listing the claims currently under examination, the Examiner failed to include Claims 57-71, which were indeed considered by the Examiner in the pending Office Action. Applicant respectfully requests a clarification on the record by the Examiner.

The Examiner acknowledged review of Applicant's Information Disclosure Statement, which Applicant mailed October 25, 2000. Applicant notes that a Supplemental Information Disclosure Statement was also mailed to the USPTO on May 23, 2002, which the Examiner is respectfully requested to review.

Applicant notes that a Supplemental Information Disclosure Statement was mailed to the USPTO by Applicant on May 23, 2002.

The Examiner acknowledged that the specification is "enabling for a method of delivering a medicament that is transported by Ca dependent K channel to a glioma or ischemia region in the brain of a mammalian subject by administering a effective amount of NONOate compound (a group of soluble guanylyl cyclase activator belong to nitric oxide donor subclass) simultaneously with the medicament."

No claims were allowed, and the following grounds of rejection were cited.

A. Rejections based on 35 U.S.C. § 112, first paragraph

1. Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 were rejected under 35 U.S.C. § 112, first paragraph, for a purported lack of enablement. The Examiner stated the following reasons:

> **Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of delivering a medicament that is transported by Ca dependent K channel to a glioma or ischemia region in the brain of a mammalian subject by administering a effective amount of NONOate compound (a group of soluble guanylyl cyclase activator belong to nitric oxide donor subclass) simultaneously with the medicament, does not reasonably provide enablement for a method of delivering any medicament to any kind of abnormal brain region by administering any potassium channel activator. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make/use the invention commensurate in scope with these claims. . .**
>
> **. . . The nature of the invention is a method of delivering a medicament to an abnormal brain region in a mammalian subject by administering a K channel activator simultaneously with the medicament, wherein the K channel activator belongs to the soluble guanylyl cyclase activator group. The inventions also encompass a pharmaceutical composition comprising said K channel activator formulated in a pharmaceutically acceptable solution together with a medicament for delivery by intra-vascular infusion or injection into a mammal, and a kit comprising said K channel activator for enhancement of a medicament delivery to an abnormal brain region or a tumor.**
>
> **The specification discloses that by administering NS 1619, YC-1, DEA/NO or PAPA/NO (all of which are Ca dependent K channel activator), at a concentration of 39.9 μg/kg, to a rat bearing implanted glioma tumor cells (RG2), the permeability of the Ca activated K channel increases in capillaries of the malignant tumor (see page 29, lines 18-31, page 30, lines 1-14). The specification also discloses that K channel activator NS-1619 and nitric oxide**

**donor increases the permeability to the ischemic rat brain region induced by MCA occlusion (see page 33-34). The specification further discloses that the density and total area of the pinocytotic vesicles increased in tumors after bradykinin and NS-1619 treatment comparing to the normal area (see page 33, Table 1), implying transendothelial vesicular transport is a primary cellular mechanism for drug delivery to the tumor cells (see page 33, lines 21-23 and 30-31)...**

**... The method of enhancing delivery to an abnormal brain region of any medicament including DNA, protein and chemical by administering a K channel activator is also unpredictable. The specification discloses that transendothelial vesicular transport is a primary cellular mechanism for drug delivery to the tumor cells. However, not all the medicament delivery is through transendothelial vesicular transport or through Ca dependent K channel. For example, some molecules may utilize ATP dependent K channel or by simple diffusion. On the other hand, viral vectors need to utilize cell surface receptors to infect the cells. Therefore, the mere demonstration of increased permeability in tumor or ischemic brain region to horse radish peroxidase, Evan blue or α-aminoisobutyric acid does not render the increased permeability to other medicament or diagnostic agent obvious. Thus, the method of enhance delivery to an abnormal brain region of any medicament by administering a K channel activator is unpredictable.**

First, Applicant has overcome this ground of rejection by amending independent Claims 1, 48, 135 and 153 to recite ". . . an in vivo activator of *calcium-activated* potassium channel . . ." (Emphasis added). Thus, in accordance with amended Claims 1, 48, 135, and 153, an activator of soluble guanylyl cyclase is also an *in vivo* activator of *calcium-activated* potassium channel, because, as Applicant's specification teaches (e.g., at page 7, lines 8-17; at page 9, lines 12-14, and at page 14, lines 2-7), calcium-activated potassium channels are activated by a cGMP-dependent mechanism. (See, e.g., **Exhibit A:** Robertson, B.E. *et al.*, *cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells*, Am. J. Physiol. 265[Cell Physiol. 34):C299-C303 [1993] [Abstract]; **Exhibit B:** Fukao, M. *et al.*, *Cyclic GMP-dependent protein kinase activates cloned $BK_{Ca}$ channels expressed in mammalian cells by direct phosphorylation at serine 1072*, J. Biol. Chem. 274(16):10927-35 [1999][Abstract]; **Exhibit C:** Becker, E.M. *et al.*, *The vasodilator-stimulated phosphoprotein (VASP): target of YC-1 and nitric oxide effects in human and rat platelets*, J. Cardiovasc. Pharmacol. 35(3):390-97 [2000][Abstract]).

In addition, there is evidence that nitric oxide and NO donors not only activate soluble guanylyl cyclase, but also activate *calcium-activated* potassium channels directly by a cGMP-independent mechanism (see, specification, at page 7, lines 17-25). (See, e.g., **Exhibit D:** Chen, C.H. *et al.*, *Nitric oxide activates $Ca^{2+}$-activated $K^+$ channels in cultured bovine adrenal chromaffin cells*, Neurosci. Lett. 248(2):127-29 [1998][Abstract]; **Exhibit E:** Vaali, K. *et al.*,

*Relaxing effects of NO donors on guinea pig trachea in vitro are mediated by calcium-sensitive potassium channels*, J. Pharmacol. Exp. Ther. 286(1):110-14 [1998][Abstract]; **Exhibit F:** Sobey, C.G. and Faraci, F.M., *Inhibitory effect of 4-aminopyridine on responses of the basilar artery to nitric oxide*, Br. J. Pharmacol. 126(6):1437-43 [1999]; and **Exhibit G:** Kurtz, A. *et al.*, *Mode of nitric oxide action on the renal vasculature*, Acta Physiol. Scand. 168(1):41-45 [2000][Abstract]). (Copies of the references in **Exhibits A-G** were previously provided to the Examiner in an Information Disclosure Statement, which Applicant mailed October 25, 2000.)

Second, Applicant believes that the Examiner has misconstrued the nature of the claimed invention by characterizing it as "a method of delivering a medicament *that is transported by Ca dependent K channel* to a glioma or ischemi[c] region in the brain of a mammalian subject, etc. . ." (Emphasis added). Again, the Examiner misconstrued the disclosures of the specification by stating "The specification discloses that transendothelial vesicular transport is a primary cellular mechanism for drug delivery to the tumor cells. However, not all the medicament delivery is *through transendothelial vesicular transport or through Ca dependent K channel*. For example, some molecules may utilize ATP dependent K channel or by simple diffusion. On the other hand, *viral vectors need to utilize cell surface receptors to infect the cells*." (Emphasis added). Contrary to the Examiner's assertions, Applicant's specification does *not* teach that the medicant is transported by the potassium channel, but, rather, the specification teaches that potassium cation is transported, whereby *vascular permeability* to the medicant is increased. It is totally consistent with Applicant's disclosures that the medicant enter target cells by any number of mechanisms, *after* it has passed through the barrier of the microvasculature (capillaries or arterioles).

In particular, the claimed method involves the enhancement of vascular permeability, but as the specification teaches, at page 20, lines 10-11, "[t]he inventive method does not depend on any particular mechanism by increased microvascular permeability to the medicant is achieved." The specification does *not* teach that the *medicant* passes through the potassium channels, but rather that "administration of the potassium channel activator increases *potassium* flux through potassium channels in endothelial cell membranes of the

capillaries and arterioles delivering blood to abnormal brain regions and/or malignant tumors." (At page 20, lines 12-14). Thus, contrary to the Examiner's assertion, the specification teaches that *potassium cations* flow through the potassium channels.

The specification further teaches that increased *potassium flux* through potassium channels in the endothelial membranes of the capillaries and arterioles delivering blood to abnormal brain regions and/or malignant tumors is thought to result in "a loosening of tight junctions *in the microvascular epithelium* and/or *increased pinocytotic activity*, enhancing the uptake of medicants from the blood vessels." (At page 20, lines 11-16; emphasis added). At page 32, lines 24-27, and in Figure 5, the specification teaches that arrays of pinocytotic vesicles were indeed observed in *endothelial cells* of brain tumor *capillaries*. This is an observation relevant to *vascular permeability*, which in no way contradicts any other mechanisms by which a medicant actually enters a target cell, *after* penetrating from the lumenal side to the abluminal side of the microvasculature, for example, by binding to a specific surface receptor molecule on the target cell.

The Examiner has noted the specification's teaching that after infusion with bradykinin, there was also a detectable increase in the number of vesicles on the surfaces of (target) tumor cells compared to control, which implies that vesicular transport is a primary cellular mechanism for drug delivery to the tumor cells (e.g., at page 32, lines 27-31, and Figure 6). But again this teaching fails to contradict the possible importance of other mechanisms of medicant delivery into target cells, such as surface receptors, and does not bear on *increasing the permeability of the microvascular endothelium* in order to deliver the medicant (through the vascular endothelium) to an abnormal brain region or to a malignant tumor, in accordance with the claimed method.

By increasing the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region, the claimed method enables a wide variety of medicants, e.g., both therapeutic agents and diagnostic agents that have a molecular weight between about 50 Daltons and about 250 kD or a particle diameter between about 50 to 250 nanometers (see, e.g., specification, at page 22, lines 8-10), to more easily penetrate from the

lumenal side of the microvasculature to the abluminal side, so that they can be delivered selectively to, i.e., come in contact with, the target cells. The claimed invention does not relate to the particular ways in which those various medicants are taken up by those target cells.

Finally, since as the Examiner acknowledged, Applicant's specification provides working examples of enhanced vascular permeability resulting from the administration of YC-1, as well as NONOates (e.g., at page 30, lines 11-14, Figure 1; at page 31, lines 4-14, Figures 2-3), it is unclear why the Examiner has acknowledged enablement, at least for a certain claim scope as to NONOates, but not as to YC-1.

In view of Applicant's amendment to Claims 1, 48, 135, and 153, the Examiner is respectfully requested to withdraw the rejection of Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160, on this ground.

The Examiner further stated:

> **. . . The state of the art at the time of filing does not teach a method of enhanced delivery of any medicament to an abnormal brain region by administering any kind of K channel activator simultaneously with the medicament. Therefore, one skilled in the art would have turn to specification for guidance to practice the claimed inventions.**
>
> **The breath of the claims is very broad. The broadest claim is drawn to a method of delivering any kind of medicament, ranging from nucleic acids, proteins, organic or inorganic chemical compounds, and diagnostic agents, to any part of the brain having abnormal feature(s). The claim also encompasses any kind of K channel activator that activates soluble guanylyl cyclase.**
>
> **However, the guidance presented in the specification is very limited. The specification only teaches that by administering NS 1619, YC-1, DEA/NO or PAPA/NO (at a concentration of 39.9 μg/kg) to a rat bearing implanted glioma tumor (RG2), the permeability of the Ca activated K channel increases in capillaries of the malignant tumor (see page 29, lines 18-31, page 30, lines 1-14). The specification also teaches that K channel activator NS-1619 and nitric oxide donor with increases the permeability to the ischemic rat brain region induced by MCA occlusion (see page 33-34). The specification fails to demonstrate whether administering K channel activators as mentioned above to other types of brain injuries including trauma, infection and stroke (as claimed in claim 2) would increase permeability to a medicament comparing to the normal brain region. The specification also fails to show whether administering K channel activator to other types of tumor or tumors in other areas (as claimed in claims 3, 57 and 58) would increase permeability to any kind of medicament. The state of art at the time of filing does not teach that brain injuries of other types or tumors of other types would result in increased expression of Ca dependent K channels. In 2001, a year after the filing date, there is only one report that teaches human glioma cells highly express Ca dependent K channel (see Ransom and Sontheimer 2001, Journal of Neurophysiology, vol. 85: 790-803). Therefore, administering K channel activator to enhance the delivery of a medicament to any type of tumor (except glioma as shown by the specification) or any type of brain injury (except ischemia) is not well recognized in the art and unpredictable . . .**

First, Applicant notes that the MCA occlusion model described in the specification as originally filed involves blockage of the middle cerebral artery (MCA) with a silicone rubber cylinder. (See, specification, at page 28, line 25 through page 29, line 5). The consequence of MCA occlusion is thus a "stroke", i.e., a condition due to a lack of oxygen to at least a part of the brain. This category overlaps to some extent with "ischemia", which is merely a lower than normal oxygen state, and which can result from stroke, as well as from other conditions.

Therefore, at the very least, Applicant's specification is also enabling with respect to "stroke" (e.g., Claim 2), as well as ischemia and glioma.

Second, Applicant strongly disagrees with the Examiner's assertion that Applicant's disclosures in the specification as filed combined with the general knowledge in the art would fail to enable embodiments of the claimed invention with respect to abnormal brain regions other than ischemia and glioma. For example, Black (USPN 5,527,778), Kozarich *et al.* (USPN 5,268,164), and Malfroy-Camine (USPN 5,112,596), all of which were cited in Applicant's Information Disclosure Statement mailed October 25, 2000, combined with the disclosures of the above-referenced application support predictable enablement for the broad scope of the claims. The Black, Kozarich *et al.*, and Malfroy-Camine patents had indicated that bradykinin and bradykinin analogs, which subsequently, and prior to the filing date of the above-captioned application, were recognized as activators of calcium-activated potassium channel ($K_{Ca}$), are able to increase the permeability of neovasculature in abnormal brain regions and malignant tumors. The disclosures of the above-referenced application extend the previous knowledge to demonstrate the presence of a higher relative number of potassium channels in neovasculature. In addition, the specification as filed demonstrates experimentally that other $K_{Ca}$ activators, such as NS-1619, YC-1 and NONOates, behave in a manner similar to bradykinin with respect to vascular permeability (e.g., at page 29, line 15 through page 30, line 16; at page 31, lines 4-14; and at page 32, lines 1-23).

The Black, Kozarich *et al.*, and Malfroy-Camine references identified many types of relevant brain abnormalities, not only ischemia, but also traumatic injuries, meningitis, infections, lesions of AIDS, Alzheimer's and Parkinson's disease, brain abscess, multiple

sclerosis, subarachnoid hemorrhage, gliomas, metastatic brain tumors (e.g., Black, at col. 4, lines 2-9; Kozarich et al., at col.7, lines 55-63; and Malfroy-Camine, at col.3, lines 47-54). To facilitate treatment of these abnormal brain regions, bradykinin and its analogs were known to allow the passage of a wide variety of molecular weight therapeutic agents (e.g., antibiotics, adrenergic agents, catecholaminergic agents, anticonvulsants, nucleotide analogs, chemotherapeutic agents, antitrauma agents, and others) and diagnostic agents (e.g., 99Tc-glucoheptonate, gallium-EDTA, ferrous magnetic or iodinated contrasting agents). (E.g., Black, at col. 4, line 57 through col. 5, line 10; Kozarich et al., at col. 7, line 55 through col. 8, line 24; and Malfroy-Camine, at col. 3, line 55 through col. 4, line 6). Thus, contrary to the examiner's assertion, the state of the art combined with the disclosures of the above-referenced application indeed support enablement for the scope of the claims, not just with respect to ischemia and glioma.

Therefore, the Examiner is respectfully requested to withdraw the rejection of Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160, on this ground.

The Examiner also stated the following:

**. . . There are five classes of nitric oxide donor including organic nitrate compounds, iron nitrosyl compounds, S-nitrosothiol compounds, sydnonimine compounds and NONOate compounds (see Feelisch 1998, Naunyn-Schmiedeberg's Arch Pharmacology, 358: 113-122). Although all NO donors somehow produce NO-related activity when applied in biological systems, several factors affect their biological action: 1) the pathways leading to NO formation, such as enzymatic or non-enzymatic production and dependence on thiol or oxygen (see page 114, lines 1-4, and Table 1). 2) the total amount of NO generated from each of those compound determines the quality and magnitude of the biological response (see page 114, col.1, 2nd paragraph lines 1-6). 3) the redox state of the NO-related species released from each compound determines the final amount of NO generated (see page 114, 2nd col. 1st paragraph). 4) The effective amount of each of those compound used in vitro versus in vivo. These factors not only affect the effectiveness of each of those compound in a given biological system, they also affect the route of administration of said compound. For example, short-lived NO donors may have to be administered as continuous infusion rather than bolus form in order to avoid the delivery of only a short burst of NO (see page 114, 1st col. 2nd paragraph, lines 6-9).**

**The tissue specificity can also affect the effectiveness of those compounds because the extent of conversion of some of those compounds to NO would also depend on enzymatic profile of the tissue or organ. To add to the complexity, the biological effects generated by some of those compounds are not entirely mediated by NO but other metabolites or decomposition product (see page 118, 1st col. 3rd paragraph, lines 18-21). Although the specification discloses that four different soluble guanylyl cyclase activators (including two nitric oxide donor) increases permeability (through increased K channel conductance) to tumor and ischemic region of the rat brain, as Feelisch indicates that "the selection of these compound (for experiments or treatments) is not a trivial issue," the method for delivering a medicament to an abnormal brain region using any of those compounds as claimed is unpredictable. Especially because some of the NO donors cause "coronary steal," an unfavorable redistribution of**

**blood away from ischemic region (see page 115, 1st col., lines 2-3). This further complicates the method of delivering a medicament to an ischemic region in brain as the compound itself would worsen the ischemic condition.**

Applicant notes that Applicant's specification provides the skilled artisan guidance to overcome the practical difficulties raised, e.g., by the teachings of Feelisch as cited by the Examiner. For example, the specification (e.g., at page 19, lines 25 through page 20, line 1) also notes that continuous infusion over a time period may be preferred over a bolus injection and that the skilled practitioner will exercise cautious monitoring of blood pressure and other parameters to avoid adverse physiological effects. In addition, based on the disclosures of the specification and the knowledge in the art (e.g., Feelisch), the skilled artisan could select a NO donor or other soluble guanylyl cyclase activator appropriate for the intended purpose, whether to treat ischemia, another brain abnormality, or a malignant tumor.

Therefore, the specification provides adequate enablement, and the Examiner is respectfully requested to withdraw the rejection of Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 on this ground.

The Examiner further stated:

**...The inventions also encompass a very broad range of the dosage of K channel activator used in the method, with the broadest claim (21 and 68) ranging from 0.075 to 1500 μg/kg body mass. However, the specification discloses the effective dose for the compounds used is between 40-80 μg/kg (2.66 and 5.3 μg/kg/min for 15min). Therefore, whether the dosage as low as 0.075 or as high as 1500 μg/kg is effective in the method of enhancing medicament delivery to an abnormal brain region is unpredictable. In fact, as Feelisch indicates that "the vasodilator properties of classical NO donors limit their potential usefulness in non-cardiovascular applications where lowering of systemic blood pressure often represents an unwanted side effect" (page 114, 2nd col. 3rd paragraph, lines 9-12). In addition, in many cases, effects obtained with comparatively high doses of NO donors are opposite to those observed at lower doses (see page 115, 1st paragraph, last 6 lines). The method of delivering a medicament to an abnormal brain region by administering a K channel activator at dosage ranging from 0.075-1500, 150, 100 or 15 μg/kg is unpredictable. In view of the factors discussed above that renders the invention unpredictable, one skilled in the art would have to turn to art or specification for guidance to practice the invention. However, the prior art does not teach a method of delivering medicament to an abnormal brain region by administering K channel activator. The specification fails to provide guidance to overcome the problems that render the inventions unpredictable. Therefore, one skill in the art would require undue amount of experimentation to practice the claimed invention commensurate in scope with these claims.**

Applicant strongly disagrees that the specification provides insufficient guidance to the skilled artisan with respect to an effective dose of an in vivo activator of calcium-activated potassium channel. The dose range recited in Claims 21 and 68 (0.075 to 1500 μg/kg body mass) is intended to encompass effective doses for any mammal ranging from small to large mammals. (See, e.g., specification, at page 13, lines 25-27). But, the specification describes more detailed preferred dose ranges for human patients, i.e., 0.075 to 150 micrograms per kilogram body mass (at page 19, lines 17-18) and for particular activators, such as YC-1, nitric oxide, and NO donors (e.g., at page 14, lines 23-25; at page 19, lines 20-22). Further, the specification states (at page 19, line 18 through page 20, line 1):

> . . .As the skilled practitioner is aware, *the physiological responses of individual patients to treatment with particular potassium channel activators will vary.* For example, generally an effective amount of YC-1 for humans is about 15 to about 45 micrograms per kg body mass, and for nitric oxide donors generally about 15 to about 45 micrograms per kg body mass. However, *the optimal amount for each individual for any particular potassium channel activator can be determined by routine means involving close physiological monitoring over the delivery period.*
>
> The dose can be administered in a bolus injection, but is preferably administered by infusion over a period of one to thirty minutes, and most preferably during a period of one to fifteen minutes. For example, in rats, a dose rate of about 0.75 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ is most suitable. *At dose rates above about 100 $\mu g\ kg^{-1}\ min^{-1}$ a concomitant fall in blood pressure has sometimes been observed. In humans, effective dose rates are about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$, with cautious monitoring of blood pressure being advised.* The practitioner skilled in the art is also cautious in regulating the total infusion volume, rate of liquid infusion, and electrolyte balance to avoid adverse physiological effects related to these. (Emphasis added).

Thus, the specification teaches that individual patients will respond differently to particular activators (as occurs with virtually any drug). The specification expressly recognizes that a fall in blood pressure is sometimes observed at higher doses of some activators, and teaches that the optimal amount for *each individual* for any particular potassium channel activator can be determined by routine means involving *close physiological monitoring* over the delivery period, "with cautious monitoring of blood pressure being advised." With the guidance provided by Applicant's specification and the general knowledge available to the skilled artisan, it would not require undue experimentation to successfully practice the claimed invention while optimizing the dose for a particular mammalian subject.

Therefore, Applicant respectfully requests the Examiner to withdraw the rejection on this ground.

2. Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner also stated the following:

> **. . .The claims recite "administering to a mammalian subject ... a potassium channel activator, said potassium activator being an activator of soluble guanylyl cyclase." The specification discloses six K channel activators (NS1619, minoxidil sulfate, bradykinin, YC-1, DEA/NO and PAPA/NO) that increase the permeability of blood tumor barrier. However, not all of the K channel activators are also activators of soluble guanylyl cyclase. For example, minoxidil sulfate activates K[ATP] channel instead of Ca dependent K channel that is responsive to cyclic GMP regulation. The specification fails to describe a structure/function relationship between K channel activators that also activates soluble guanylyl cyclase, for instance, what common structures or characteristics that are shared by those compounds which allow them to activate both K channel and soluble guanylyl cyclase? However, applicants are broadly claiming any K channel activator being an activator of soluble guanylyl cyclase. Therefore, the specification fails to describe the claimed subject matter in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.**

Applicant agrees with the Examiner that not all potassium channel activators are also activators of soluble guanylyl cyclase, e.g., minoxidil sulfate activates ATP-sensitive potassium channels (KATP), which unlike calcium-activated potassium channels are unresponsive to cyclic GMP. In response Applicant has overcome this ground of rejection by amending independent Claims 1, 48, 135 and 153 to recite ". . . an in vivo activator of *calcium-activated* potassium channel . . ." (Emphasis added). Thus, in accordance with amended Claims 1, 48, 135, and 153, an activator of soluble guanylyl cyclase is also an *in vivo* activator of *calcium-activated* potassium channel, because, as Applicant's specification teaches (e.g., at page 7, lines 8-17; at page 9, lines 12-14, and at page 14, lines 2-7), calcium-activated potassium channels are activated by a cGMP-dependent mechanism. (See, e.g., **Exhibit A:** Robertson, B.E. *et al.*, *cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells*, Am. J. Physiol. 265[Cell Physiol. 34):C299-C303 [1993] [Abstract]; **Exhibit B:** Fukao, M. *et al.*, *Cyclic GMP-*

*dependent protein kinase activates cloned $BK_{Ca}$ channels expressed in mammalian cells by direct phosphorylation at serine 1072*, J. Biol. Chem. 274(16):10927-35 [1999][Abstract]; **Exhibit C:** Becker, E.M. *et al.*, *The vasodilator-stimulated phosphoprotein (VASP): target of YC-1 and nitric oxide effects in human and rat platelets*, J. Cardiovasc. Pharmacol. 35(3):390-97 [2000][Abstract]).

In addition, there is evidence that nitric oxide and NO donors not only activate soluble guanylyl cyclase, but also activate *calcium-activated* potassium channels directly by a cGMP-independent mechanism (see, specification, at page 7, lines 17-25). (See, e.g., **Exhibit D:** Chen, C.H. *et al.*, *Nitric oxide activates $Ca^{2+}$-activated $K^+$ channels in cultured bovine adrenal chromaffin cells*, Neurosci. Lett. 248(2):127-29 [1998][Abstract]; **Exhibit E:** Vaali, K. *et al.*, *Relaxing effects of NO donors on guinea pig trachea in vitro are mediated by calcium-sensitive potassium channels*, J. Pharmacol. Exp. Ther. 286(1):110-14 [1998][Abstract]; **Exhibit F:** Sobey, C.G. and Faraci, F.M., *Inhibitory effect of 4-aminopyridine on responses of the basilar artery to nitric oxide*, Br. J. Pharmacol. 126(6):1437-43 [1999]; and **Exhibit G:** Kurtz, A. *et al.*, *Mode of nitric oxide action on the renal vasculature*, Acta Physiol. Scand. 168(1):41-45 [2000][Abstract]). (Copies of the references in **Exhibits A-G** were previously provided to the Examiner in an Information Disclosure Statement, which Applicant mailed October 25, 2000.)

Consequently, Applicant's specification as originally filed describes a structure/function relationship between activators of calcium-activated potassium channel activators and activators of soluble guanylyl cyclase, in accordance with amended Claims 1, 48, 135, and 153.

Therefore, in view of Applicant's amendment to Claims 1, 48, 135, and 153, the Examiner is respectfully requested to withdraw the rejection of Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160, on this ground.

B. Rejections based on 35 U.S.C. § 112, second paragraph

Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 were rejected under 35 U.S.C. § 112, second paragraph. The Examiner stated the following reasons.

> **Regarding claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160, the word "medicant" renders the claim indefinite because the word has no meaning. It appears to be a misspelling for the word "medicament." Amending the claim as such would obviate this rejection.**

Contrary to the Examiner's assertion, the recitation of the word "medicant" is not a misspelling of the word "medicament." Rather, Applicant deliberately used the word "medicant" to include *both* therapeutic *and* diagnostic agents, as opposed to a "medicament", the dictionary meaning of which is limited to *therapeutic agents* that are used to treat, prevent, or alleviate the symptoms of disease. (See, e.g., **Exhibit H**: WordNet 1.7 Vocabulary Helper: medicament, 7/3/2002).

First, the word "medicant" is not unknown, as illustrated by at least four pre-filing date references that Applicant has appended as **Exhibit I**, in which the expression "*medicant*" or "pre-*medicant*" is used. (**Exhibit I:** Purnell WD, Gregg JM, Ann Ophthalmol 7(7):921-3 [1975]; Hughes BL et al., Poult Sci 70(3):476-82 [1991]; Badcock NR et al., Eur J Clin Pharmacol 38(2):153-5 [1990]; Sobti VK et al., Zentralbl Veterinarmed A 37(3):170-3 [1990]).

Regardless, it is well established that a patent applicant is permitted to be his own lexicographer. Applicant respectfully brings the Examiner's attention to the specification, e.g., at page 21, line 8 through page 22, line 10, where "medicants" are stated to include (1) drugs, i.e., chemotherapeutic agents and therapeutic viral particles, and (2) *diagnostic agents*.

Thus, the word "medicant" is not meaningless, and consequently, Claims 1-10, 12-24, 48-55, 57-71, 135-144 and 146-160 are not rendered indefinite.

Therefore, the Examiner is respectfully requested to withdraw the rejection on this ground.

The Examiner also stated:

> **. . . Regarding claims 1-10, 12-24, 48-55 and 57-71, the term "substantially simultaneously" renders the claim indefinite because it is unclear whether it is simultaneous or not. As such, the metes and bounds of the claims cannot be established.**

Applicant strongly disagrees with the Examiner's assertion, because Applicant's specification clearly states the meaning of the phrase "substantially simultaneously." At page 20, line 30 through page 21, line 5, the specification states "'[s]ubstantially simultaneously' means that the medicant is administered within about one hour after the potassium channel activator is last administered . . . and most preferably, is administered simultaneously with the potassium channel activator . . ." or ". . . within about 30 minutes before . . . the potassium channel activator is first administered." Thus, the specification sets definite metes and bounds to the expression "substantially simultaneously."

Therefore, the Examiner is respectfully requested to withdraw the rejection on this ground.

The Examiner further stated the following:

> **. . . Regarding claims 17, 64 and 150, the word "derived" renders the claims indefinite because the nature and derivative process is not known. As such, the metes and bounds of the claims cannot be established.**

Applicant has amended Claims 17, 64, and 150 for greater clarity to delete the phrases "adenovirus-derived" and "herpes simplex-derived", and instead to recite the phrase "wherein the viral vector is a therapeutic adenoviral vector or herpes simplex virus vector." The amendments to Claims 17, 64, and 150 are supported by the specification as originally filed, e.g., at page 21, line 30 through page 22, line 1. The amendments are to clarify that as long as the adenoviral or HSV vectors are therapeutic vectors, the particular biochemical nature of the "derivative", or the molecular process by which it occurred, are not important for purposes of the claimed invention.

For example, an adenoviral particle or HSV particle might be pseudotyped with foreign coat proteins and might contain any number of possible genetic insertions and/or deletions to

produce a therapeutic vector suitable for treating one disease condition or another, but the particular biochemical nature of the therapeutic adenoviral or HSV "derivative" is not relevant to the claimed invention, because it is the *particle size* that matters. The skilled artisan would know that a modified adenoviral or HSV vector would have a particle size comparable to the native adenoviral particle or HSV particle, regardless of the precise derivative modifications made to the viral particle to produce the vector for its intended therapeutic purpose. As long as the particle diameter is "between about 50 to 250 nanometers" (see, specification, at page 22, lines 8-10), the permeability to the viral vector of a capillary or arteriole delivering blood to cells of an abnormal brain region will be increased, in accordance with the method claimed in Claims 17 and 64.

The amendments to Claims 17, 64, and 150 overcome the rejection, which the Examiner is respectfully requested to withdraw.

## CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is now ready for allowance. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney at (213) 896-6665.

Respectfully submitted,

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**Version With Markings to Show Changes Made**

Deletions are indicated with **bold** brackets to distinguish them from brackets that are part of the desired text.

In the Specification:

Please delete the paragraph at page 4, lines 11-17, and insert therefor the following:

--Intracarotid infusion of leukotriene C**[**.sub.4**]**$_4$(LTC$_4$**[**.sub.4**]**) selectively increases the permeability in brain tumor capillaries without affecting the permeability in normal brain capillaries. The effect of LTC$_4$**[**.sub.4**]** on brain tumor capillaries is, however, limited to small molecules and it can only slightly increase the permeability of those small molecules in abnormal brain tissue relative to normal. Accordingly, LTC$_4$**[**.sub.4**]** does not significantly increase the delivery of some larger water soluble molecules to brain tumors or other abnormalities.--.

Please delete the paragraph at page 4, line 18 through page 5, line 3, and insert therefor the following:

--The vasoactive n**[**a**]**onopeptide bradykinin and agonists or polypeptide analogs thereof (e.g., receptor-mediated permeabilizers [RMPs]) have been injected intravenously to increase blood-brain barrier permeability to co-administered neuropharmaceutical or diagnostic agents. (B. Malfroy-Camine, *Method for increasing blood-brain barrier permeability by administering a bradykinin agonist of blood-brain barrier permeability*, U.S. Patent No. 5,112,596; J.W. Kozarich *et al.*, *Increasing blood brain barrier permeability with permeabilizer peptides*, U.S. Patent No. 5,268,164). Intracarotid infusion of bradykinin will selectively increase permeability 2- to 12-fold in brain tumor and ischemic brain capillaries for molecules ranging in molecular weight from 100 to 70,000 Daltons (Inamura, T. *et a l.*, Bradykinin selectively opens blood-

tumor barrier in experimental brain tumors, J. Cereb. Blood Flow Metab. 14(5):862-70 [1994]). Bradykinin does not increase permeability in the normal blood brain barrier except at very high doses. (Wirth, K. *et al.*, *DesArg9-D-Arg[Hyp3,Thi5,D-Tic7,Oic8]bradykinin (desArg10-[Hoe140]) is a potent bradykinin B1 receptor antagonist*, Eur. J. Pharmacol. 205(2):217-18 [1991]). Opening of the blood-tumor barrier by bradykinin is transient, lasting 15 to 20 minutes. (Inamura *et al.* [1994]). After opening of abnormal brain capillaries with bradykinin, the capillaries become refractory to the bradykinin effect for up to 60 minutes. (Inamura *et al.* [1994]).--.

Please delete the paragraph at page 19, lines 3-11, and insert therefor the following:

--However, the potassium channel activator employed in the inventive methods is one other than the vasodilator bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)(SEQ ID NO:1), or a polypeptide bradykinin analog, such as receptor mediated permeabilizer (RMP)-7 or A7 (e.g., Kozarich *et al.*, U.S. Patent No. 5,268,164 and PCT Application No. WO 92/18529). Other analogs of bradykinin include related peptide structures which exhibit the same properties as bradykinin but have modified amino acids or peptide extensions on either terminal end of the peptide. Examples of bradykinin analogs include [phe$^{8}$[.sup.8] ($CH_2$[.sub.2 ]–NH) Arg$^{9}$[.sup.9 ]-bradykinin, N–acetyl [phe$^{8}$[.sup.8] ($CH_2$[.sub.2 ]--NH--Arg$^{9}$[.sup.9 ]] bradykinin and desArg9-bradykinin.--.

In the Claims:

Please amend Claims 1, 17- 24, 48, 64-71, 135-137, 150, and 153, and add new Claims 162-189 as follows.

1.(Twice Amended) A method of delivering a medicant to an abnormal brain region in a mammalian subject, comprising:

administering to a mammalian subject having an abnormal brain region an in vivo activator of calcium-activated potassium channel[ activator], said [potassium channel] activator being an activator of soluble guanylyl cyclase, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region; and

administering the medicant to the subject, simultaneously or substantially simultaneously with the [potassium channel] activator[ the medicant], so that the medicant is delivered selectively to the cells of the abnormal brain region compared to normal brain regions.

17.(Amended) The method of Claim 13, wherein the viral vector is a[n] therapeutic adenoviral[us-derived] vector or herpes simplex virus[-derived] vector.

18.(Amended) The method of Claim 1, wherein administering the [potassium channel] activator is by intravenous or intra-arterial infusion or injection.

19.(Amended) The method of Claim 1, wherein administering the [potassium channel] activator is by intracarotid infusion or injection.

20.(Amended) The method of Claim 1, wherein the [potassium channel] activator is administered to the mammalian subject by a bolus injection.

21.(Amended) The method of Claim 1, wherein the [potassium channel] activator is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

22.(Amended) The method of Claim 21, wherein the [potassium channel] activator is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

23.(Amended) The method of Claim 1, wherein the [potassium channel] activator is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

24.(Amended) The method of Claim 23, wherein the [potassium channel] activator is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

48.(Twice Amended) A method of delivering a medicant to a malignant tumor in a mammalian subject, comprising:

administering to a mammalian subject having a malignant tumor an in vivo activator of calcium-activated potassium channel[ activator], said [potassium channel] activator being an activator of soluble guanylyl cyclase, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the malignant tumor; and

administering the medicant to the subject, simultaneously or substantially simultaneously with the [potassium channel] activator[ the medicant], so that the medicant is delivered selectively to the malignant cells compared to non-malignant cells.

64.(Amended) The method of Claim 60, wherein the viral vector is a[n] therapeutic adenoviral[us-derived] vector or herpes simplex virus[-derived] vector.

65.(Amended) The method of Claim 48, wherein administering the [potassium channel] activator is by intravenous or intra-arterial infusion or injection.

66.(Amended) The method of Claim 48, wherein administering the [potassium channel] activator is by intracarotid infusion or injection.

67.(Amended) The method of Claim 48, wherein the [potassium channel] activator is administered to the mammalian subject by a bolus injection.

68.(Amended) The method of Claim 48, wherein the [potassium channel] activator is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

69.(Amended) The method of Claim 68, wherein the [potassium channel] activator is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

70.(Amended) The method of Claim 48, wherein the [potassium channel] activator is administered to the mammalian subject at a dose rate of about 0.075 to about 100 $\mu g\ kg^{-1}\ min^{-1}$ for up to about 30 minutes.

71.(Amended) The method of Claim 70, wherein the [potassium channel] activator is administered to the mammalian subject at a dose rate of about 0.075 to about 15 $\mu g\ kg^{-1}\ min^{-1}$.

135. (Twice Amended) A pharmaceutical composition comprising a combination of an in vivo activator of calcium-activated potassium channel[ actvator], said [potassium channel] activator being an activator of soluble guanylyl cyclase, formulated in a pharmaceutically acceptable solution together with a medicant for delivery by intravascular infusion or injection into a mammal.

136.(Amended) The pharmaceutical composition of Claim 135, wherein the solution is formulated to deliver a dose rate of about 0.075 to 1500 micrograms of the [potassium channel] activator per kilogram body mass in a pharmaceutically acceptable fluid volume over a maximum of about thirty minutes.

137. The pharmaceutical composition of Claim 135, wherein the solution is formulated to deliver a dose rate of about 0.075 to 150 micrograms of the [potassium channel] activator per kilogram body mass in a pharmaceutically acceptable fluid volume over a period of up to thirty minutes.

150.(Amended) The method of Claim 146, wherein the viral vector is a[n] <u>therapeutic</u> adenovira<u>l</u>[us-derived] vector or herpes simplex virus[-derived] vector.

153.(Twice Amended) A kit for enhancing the delivery of a medicant to an abnormal brain region and/or to a malignant tumor, comprising:

<u>an in vivo activator of calcium-activated</u> potassium channel[ activator], said [potassium channel] activator being an activator of soluble guanylyl cyclase; and

instructions for using the [potassium channel] activator for enhancing the delivery of a medicant to an abnormal brain region or to a malignant tumor.

Please add new Claims 162-189 as follows.

--162.(New) A method of delivering a medicant to an abnormal brain region in a mammalian subject, comprising:

administering to a mammalian subject having an abnormal brain region an activator of soluble guanylyl cyclase selected from the group consisting of YC-1 and a NONOate, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region; and

administering the medicant to the subject, simultaneously or substantially simultaneously with the activator, so that the medicant is delivered selectively to the cells of the abnormal brain region compared to normal brain regions.

163.(New) The method of Claim 162, wherein the NONOate is diethylamine-NONOate, diethylene triamine-NONOate, dipropylenetriamine-NONOate, spermine-NONOate, propylamino-propylamine-NONOate, MAHMA-NONOate, piperazi-NONOate, proli-NONOate, sulfo-NONOate, Angelis salt, or sulfite NONOate.

164.(New) The method of Claim 162, wherein administering the activator is by intravenous or intra-arterial infusion or injection.

165.(New) The method of Claim 162, wherein administering the activator is by intracarotid infusion or injection.

166.(New) The method of Claim 162, wherein the activator is administered to the mammalian subject by a bolus injection.

167.(New) The method of Claim 162, wherein the activator is administered to the mammalian subject in an amount from about 0.075 to 1500 micrograms per kilogram body mass.

168.(New) The method of Claim 167, wherein the activator is administered to the subject in an amount from about 0.075 to 150 micrograms per kilogram body mass.

169.(New) The method of Claim 162, wherein the activator is administered to the mammalian subject at a dose rate of about 0.075 to about 100 μg kg-1 min-1 for up to about 30 minutes.

170.(New) The method of Claim 169, wherein the activator is administered to the mammalian subject at a dose rate of about 0.075 to about 15 μg kg-1 min-1 for up to about 30 minutes.

171.(New) The method of Claim 162, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

172.(New) The method of Claim 162, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

173.(New) The method of Claim 172, wherein the diagnostic agent is an imaging or contrast agent.

174.(New) The method of Claim 172, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

175.(New) The method of Claim 162, wherein the medicant is a N-methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

176.(New) The method of Claim 175, wherein the viral vector is a therapeutic adenoviral or herpes simplex virus vector.

177.(New) The method of Claim 162, wherein the abnormal brain region is a glioma or ischemic brain region.

178.(New) A method of delivering a medicant to an abnormal brain region in a mammalian subject, comprising:

administering to a mammalian subject having an abnormal brain region a NONOate, under conditions and in an amount sufficient to increase the permeability to the medicant of a capillary or arteriole delivering blood to cells of the abnormal brain region; and

administering the medicant to the subject, simultaneously or substantially simultaneously with the NONOate, so that the medicant is delivered selectively to the cells of the abnormal brain region compared to normal brain regions.

179.(New) The method of Claim 178, wherein the NONOate is diethylamine-NONOate, diethylene triamine-NONOate, dipropylenetriamine-NONOate, spermine-NONOate, propylamino-propylamine-NONOate, MAHMA-NONOate, piperazi-NONOate, proli-NONOate, sulfo-NONOate, Angelis salt, or sulfite NONOate.

180.(New) The method of Claim 178, wherein administering the NONOate is by intravenous or intra-arterial infusion or injection.

181.(New) The method of Claim 178, wherein administering the NONOate is by intracarotid infusion or injection.

182.(New) The method of Claim 178, wherein the NONOate is administered to the mammalian subject by a bolus injection.

183.(New) The method of Claim 178, wherein said mammal is a human, non-human primate, canine, feline, bovine, porcine, ovine, mouse, rat, gerbil, hamster, or rabbit.

184.(New) The method of Claim 178, wherein the medicant is a therapeutic cytotoxic agent, DNA expression vector, viral vector, protein, oligonucleotide, nucleotide analog, antimicrobial agent, interferon, cytokine, cytokine agonist, cytokine antagonist, immunotoxin, immunosuppressant, boron compound, monoclonal antibody, adrenergic agent, anticonvulsant, ischemia-protective agent, anti-trauma agent, anticancer chemotherapeutic agent, or diagnostic agent.

185.(New) The method of Claim 184, wherein the diagnostic agent is an imaging or contrast agent.

186.(New) The method of Claim 184, wherein the diagnostic agent is a radioactively labeled substance, a gallium-labeled substance, or a contrast agent selected from the group consisting of ferrous magnetic, fluorescent, luminescent, and iodinated contrast agents.

187.(New) The method of Claim 178, wherein the medicant is a N-methyl-D-aspartate (NMDA) receptor antagonist, antibiotic, interleukin-2; or transforming growth factor-β, cisplatin, carboplatin, tumor necrosis factor-α, methotrexate, 5-fluorouracil, amphotericin, daunorubicin, doxorubicin, vincristine, vinblastine, busulfan, chlorambucil, cyclophosphamide, melphalan, or ethyl ethanesulfonic acid.

188.(New) The method of Claim 187, wherein the viral vector is a therapeutic adenoviral or herpes simplex virus vector.

189.(New) The method of Claim 178, wherein the abnormal brain region is a glioma or ischemic brain region.--.